Support for the amendment to claim 4 can be found on page 8, lines 27-29, of the specification. No new matter has been added by this amendment to the claims.

Claims 1-7 were rejected variously for indefiniteness, lack of enablement, and obviousness. Reconsideration and withdrawal of the rejections to the claims in view of the following remarks is respectfully requested.

1. The Claims are definite under 35 USC § 112, second paragraph.

Claim 6 remains rejected as the term "overexpressed" is deemed indefinite. Applicants respectfully submit that one of ordinary skill in the art would understand the common meaning of "overexpressed". Claim 6 states that "heparinase enzyme is overexpressed from a recombinant nucleotide sequence in Flavobacterium heparinum." As one of ordinary skill in the art would be aware from Applicants' specification, the bacterium Flavobacterium heparinum naturally expresses heparinase enzyme. As one of ordinary skill in the art would understand and as is appropriately recited in claim 6, the term "overexpressed" is a term used in genetic engineering means that heparinase enzyme is expressed in the bacterium at a level that is greater than the standard level of expression. As previously stated, the commonly understood meaning of "overexpressed" by those of ordinary skill in the art and Applicants teachings in the specification would apprise one of ordinary skill in the art of the scope of Claim 6. See, for example, page 8 and the teachings of the detailed description beginning on page 13.

Claims 1-7 remain rejected as the term "heparinase enzyme" is deemed indefinite. The Examiner specifically requests a statement as to whether or not the term "heparinase enzyme" encompasses platelet heparitinase taught by Vlodavsky et al. Applicants respectfully submit that page 8, lines 10-19 of the specification teach

that enzymes which degrade heparin and heparan sulfate moieties are referred to in the specification as "heparinase" or "heparinase enzyme", and that heparin and heparan sulfate moieties are degraded on the surface of endothelial cells and from basement membranes by administration of heparinase enzyme. Thus, as platelet heparitinase has been shown to degrade heparin or heparan sulfate, it is a heparinase enzyme.

Applicants respectfully submit that claims 1-7 are definite. Accordingly, Applicants request reconsideration and withdrawal of these rejections.

2. Claims 1-7 are enabled under 35 USC §112, first paragraph.

Claims 1-7 remain rejected as lacking enablement. In the Office Action at paragraph 7, it is asserted that "the specification, while being enabling for a method to decrease inflammatory response in ischemic tissue, does not reasonably provide enablement for numerous inflammatory diseases or conditions disclosed on page 1, lines 25-35" of the specification.

Applicants respectfully submit that, as taught in Applicants specification at least at page 8, lines 18-35, the administration of heparinase enzymes to a patient results in the degradation of heparin and heparan sulfate moieties on the surface of endothelial cells and from basement membranes. The removal of the heparin and heparan sulfate moieties from the surface of activated endothelial cells and from their basement membrane causes chemokines, which are bound to heparin and heparan sulfate, to be released from the endothelial cell surfaces and basement membrane. The loss of bound chemokines decreases the localized concentration of chemokines and disrupts the chemokine gradient produced by activated endothelium, thereby inhibiting chemokine activation of rolling leukocytes, which is required for firm

adhesion, and preventing extravasation of leukocytes along the chemokine gradient. Thus, administration of heparinase enzyme decreases localized tissue inflammation by interfering with a fundamental mechanism of leukocyte recruitment. These features of the method of the invention are recited in claims 1-5. This teaching of the specification is validated by the Examples of the specification, as briefly summarized below.

In the specification, Applicants provide data from *in vitro* and *in vivo* model systems that are accepted model systems for studying localized inflammation. The results presented in Example 1 and 3 demonstrate that the administration of heparinase enzyme in an *in vitro* assay system degrades heparan and heparin sulfate releasing chemokines from activated endothelium (endothelial cells which express chemokines on their cell surface due to exposure to cytokines and chemoattractants which are secreted by inflamed tissue). The treatment of activated endothelium with heparinase enzyme was also demonstrated to inhibit neutrophil adhesion and neutrophil extravasation in accepted *in vitro* model systems for adhesion and extravasation, see Examples 4, 5 and 6. The treatment of activated endothelium *in vivo*, through the administration of heparinase enzyme to vasculature that had been injured by ischemia, demonstrated that the number of leukocytes adhered to the vasculature did not significantly increase above normal levels. This result is in direct contrast to the large number of leukocytes that were adhered to the vascular endothelium in animals that had not been treated with heparinase enzyme, see Example 7 and Figure 12. As would be expected from this result, the number of extravasated leukocytes did not significantly change during reperfusion of the ischemia injured tissue which had been treated with heparinase enzyme whereas the number of extravasated leukocytes in untreated tissue which had been injured by ischemia was significantly greater, see Example 7 and Figure 14. In Example 8,

Applicants demonstrate, in an *in vivo* model of myocardial infarction, that administration of heparinase enzymes either before or after coronary occlusion attenuates the extent of myocardial tissue necrosis that would otherwise result from a localized inflammatory response subsequent to myocardial infarction.

The teachings of the Examples taken together demonstrate that Applicants have shown that the method of the invention will be useful to decrease the localized inflammatory response in any tissue in which the localized inflammation arises from leukocyte recruitment to a chemokine gradient.

In the Office Action, the Examiner agrees that Applicants have demonstrated that the method of the invention has been enabled for the treatment of ischemia/ reperfusion injuries, but questions whether or not other inflammatory diseases or conditions have been enabled. On page 1 of the specification, Applicants listed several conditions in which a detrimental inflammatory response can occur. Ischemia/reperfusion injury can occur following myocardial infarction, shock, stroke, organ transplantation or cardiopulmonary by-pass surgery, and can lead to allograft rejection. In each of rheumatoid arthritis, antigen induced asthma, and allergic rhinitis there is a selective accumulation and activation of leukocytes as one part of these allergic diseases. As detailed above, localized inflammatory responses arise from a common mechanism of action, and Applicants' invention is a method directed to decreasing the inflammatory response which results from such common mechanism.

To support the enablement of the claim method to additional inflammatory diseases or conditions, Applicants submit herewith a copy of a recent review article, Luster, A. D., 1998, *New Engl. J. Med.*, 338:436-445 ("Luster"), which reviews the role of chemokines in several inflammatory diseases. In this article, Luster states that

chemokines control the process by which leukocytes are attracted to tissue and notes that chemokines control an essential step in the inflammation process. Luster shows on page 439, col. 2 through page 441, that chemokines have been detected in a wide variety of diseased tissues (see, Fig. 3). These inflammatory diseases or conditions include those mentioned in the specification, namely, asthma, glomerulonephritis and rheumatoid arthritis, as well as several other diseases or conditions. As discussed by Luster, tissue infiltration by lymphocytes and macrophages occurs in many chronic diseases. Studies of such diseased tissues has revealed that chemokine levels are increased in the diseased tissues when compared to normal tissues. In some cases chemokine levels have been directly correlated with the number of activated T lymphocytes found in the diseased tissue, and in other cases chemokines are found to be present in the diseased tissue when they are not present in the normal tissue. These findings directly corroborate the teachings of the specification and the results presented in the Examples, and further support that Applicants' method is fully enabled.

Applicants respectfully submit that, the claimed invention of decreasing inflammatory responses in a patient's tissue by administering an effective amount of heparinase enzyme is fully enabled based on Applicants' teaching in the specification that such administration results in the release of bound chemokines from endothelial cells, thus inhibiting leukocyte rolling on endothelium and leukoycyte extravasation, and decreasing the accumulation of leukocytes in tissue adjacent to endothelial cell surfaces and extracellular matrices, all as claimed in pending claims 1-5. Luster's review article confirms that one of ordinary skill in the art would expect that the method of the invention would be useful to decrease localized inflammation that

arises from a wide range of conditions or diseases, including chronic conditions and diseases.[1]

Based on the foregoing remarks, the Examiner is respectfully requested to reconsider and withdraw this rejection of claims 1-7.

3. The claims are patentable under 35 USC § 103 in view of the cited art.

Claims 1-7 were rejected under 35 USC §103(a) as being unpatentable over Hoogewerf et al., Gilat et al. (J. Exp. Med.), Vlodavsky et al., Zimmerman et al. (U.S. 5,169,772), Fuks et al. (U.S. 5,362,641), and Sasisekharan et al. (U.S. 5,567,417) in view of Lider et al., Ratner et al. or Gilat et al. (J. Immunol.). On page 6 of the Office Action it is stated that the rejection under §103(a) has been modified in response to Applicants response in Paper 6. Applicants respectfully traverse this new rejection of Claims 1-7.

Attached hereto is a Declaration under Rule 1.131, signed by the inventors of the pending claims, which Declaration establishes that the claimed invention was conceived and reduced to practice at least as early as December 1994, and prior to the publication of the primary references Hoogewerf et al. (J. Biol. Chem., published February 1995) and Gilat et al. (J. Exp. Med., published May 1995), and the secondary references Lider et al. (Proc. Natl. Acad. Sci., USA, published May 1995), and Gilat et al. (J. Immunol., published December 31, 1994). Accordingly these references should be removed from the rejection, and the following remarks do not address these references.

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[1]Applicants would be pleased to submit the Luster review article with a Rule 1.132 Declaration if the Examiner would deem it appropriate.

As acknowledged by the Examiner, none of the remaining primary references, Vlodavsky et al., Zimmerman et al., Fuks et al., and Sasisekharan et al. teach or suggest the use of heparinase enzymes to decrease a localized inflammatory response.

The teaching of the remaining secondary reference, Ratner et al., is stated to provide motivation to one of skill in the art "to administer heparinase to patients with the expectation that heparinase would degrade heparin at the site of inflammation thus leading to T cell movement through the basement membranes for the reasons taught by Ratner et al." (see pg. 82). Applicants respectfully submit, however, that this teaching of Ratner et al. actually *teaches away* from the claimed invention. Ratner et al. teaches that administration of heparanases leads to T cell movement through the basement membrane, suggesting to one of skill in the art that administration of heparanase enzymes would actually increase the inflammatory response rather than decrease the inflammatory response. As taught in the instant specification, the movement of T cells (leukocytes) through the basement membrane is a cause of the localized inflammatory response. Therefore, Ratner et al. does not provide motivation for one of skill in the art to administer heparinase enzymes to a patient in order to decrease the localized inflammatory response, as recited in Claim 1.

Based on the foregoing remarks, the Examiner is respectfully requested to reconsider and withdraw this rejection of claims 1-7.

5. Obviousness-Type Double Patenting.

Claims 1-7 remain provisionally rejected under the judicially created doctrine of obvious-type double patenting over claims 1-10 of copending Application No. 08/273,109. Applicants respectfully request that this rejection be withdrawn until allowance of patentably indistinct subject matter in both the instant application and in Application No. 08/273,109.

Summary

Entry of the present amendments, the Rule 131 Declaration, and reconsideration of the amended application, in view of the foregoing remarks, are respectfully requested. The amended application should be in condition for allowance, and such action is respectfully requested. If the Examiner believes that a telephone conversation would expedite prosecution in this Application, the Examiner is invited to telephone the undersigned at (617) 526-6460.

If there are any additional payments due or credits owed, please make them to our Deposit Account No. 08-0219.

Respectfully submitted,
HALE AND DORR, LLP

Dated: November 20, 1998

Gretchen A. Rice

Gretchen A. Rice, Ph.D.
Reg. No. 37,429
Attorney for Applicants

Hale and Dorr, LLP
60 State Street
Boston, MA 02109
rice/104385.140/11_98resp.wpf